Pre-Ph.D. Registration Synopsis

STUDIES ON ANTIDIABETIC, ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITIES OF SYNTHESIZED SILVER NANOPARTICLES FROM LEAF EXTRACTS OF TWO PLANTS OF VERBENACEAE FAMILY (*Vitex negundo* Linn. AND *Vitex peduncularis* Wall. ex Schauer)

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STUDIES ON ANTIDIABETIC, ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITIES OF SYNTHESIZED SILVER NANOPARTICLES FROM LEAF EXTRACTS OF TWO PLANTS OF VERBENACEAE FAMILY (*Vitex negundo* Linn. AND *Vitex peduncularis* Wall. ex Schauer)

STATE OF ART

In the 21st century, nanotechnology is emerging as an advantageous technology. Nanotechnology has the potential to change the nanoscience theory into valuable applications by observing, measuring, manipulating, assembling, controlling, and manufacturing nanomaterials[1] .The size of the nanomaterial or nanoparticle is measured at the nanoscale, which deals with dimensions between 1 - 100nm[2].

Currently , nanoparticles are produced biologically , and this is considered an emerging field of nanotechnology . Nanoparticles that are produced by biological systems are called bionanoparticles (BNP)[3]. Nanoparticles adaptable nature and better performance make them more suitable for nanotechnological applications. Nanoparticles are synthesized by reducing metal ions into uncharged metallic nanoparticles. During the synthesis of nanoparticles, various harmful chemicals are used as reducing agents, which are toxic to the environment . Therefore, to reduce the use of these toxic chemicals, there is the development of green technology that uses natural resources, which is an eco-friendly method for the synthesis of nanoparticles[4].

In the present scientific world, green synthesis methods are used to produce nanoparticles biologically from the extracts of various parts of plants , algae , fungi, and microorganisms . The green synthesis method is eco-friendly , non toxic, and cost-effective; therefore, the use of the green method is more frequent than the conventional physical and chemical methods[5]. The application of metal nanoparticles is more significant in various scientific domains ; therefore, many research groups are working for the advancement of the green method for the synthesis of metal nanoparticles[6] . The application of nanoparticles is classified into two types, i.e., biological application and non biological application. Biological applications include the use of nanoparticles in anti-viral[8] , anti-bacterial[7] , anti-fungal[9] , anti-oxidant[8], anti-cancer[12], anti-inflammatory[8], and anti-diabetic activities[8] .Non biological applications include the use of nanoparticles in dye -sensitized solar cells (DSSCs)[11] , photocatalysis of the harmful dye methylene blue, reduction of p- nitrophenol (4- nitrophenol), and its derivatives used in pesticides[10].

The World Health Organization (WHO) has recognized the use of medicinal plants in treating a variety of disorders, including diabetes mellitus (DM), cancer, HIV infection, tuberculosis (TB), and malaria.Conventional medicine, which makes use of therapeutic plants, plays an essential role in healthcare, especially in developing countries where access to conventional medicine might be limited. The WHO supports integrating traditional medicine into national health systems and encourages the study and validation of medicinal plants to ensure their safety and efficacy. However, it is essential

to approach the use of medicinal plants with caution and relay on scientific evidence to support their use in treating these conditions[12].

Diabetes mellitus (DM) and its associated complications have high incidence , morbidity, and mortality rates; therefore, it is currently a severe public health concern. Diabetes mellitus is a chronic hyperglycemia. It is an irreversible disease that is caused by altered carbohydrate, lipid, and protein metabolism[13]. Diabetes mellitus is a condition where the blood glucose level is high due to a deficiency of the insulin hormone or, if insulin is available, is not working properly . Hyperglycemia is a syndrome that may leads to polyuria , polyphagia , polydipsia , blurred vision , and weight loss in diabetic patients. The main cause of altered carbohydrate, fat, and protein metabolism is that the available insulin action on target tissues is not working effectively [14]. Diabetes mellitus is of three types , i.e., (i) type 1 diabetes mellitus (T1DM) , (ii) type 2 diabetes mellitus (T2DM), and (iii) gestational diabetes mellitus (GDM) . The incidence rate of type 2 diabetes is higher when compared to type 1 and gestational diabetes . The incidence of type 2 diabetes is indeed high and continues to rise globally. In T2DM, there is a reduction in glucose tolerance because of insulin resistance. T2DM affects approximately 460 million people worldwide because its prevalence rate is high , i.e., about 90%. Whereas the T1DM annual incidence rate is 2% to 5% . T1DM is a most prevalent pediatric illness , and it is caused by the autoimmune destruction of pancreatic beta cells that produce insulin. Gestational diabetes mellitus (GDM) is a hyperglycemic condition related to glucose intolerance that develops during pregnancy[28] .There are various methods to treat diabetes , but plant -based drugs are thought to be less toxic , effective, and have no side effects. A drugs bioavailability can play a vital role in the treatment of diseases. If the drug molecule is complex, its absorption is decreased, which limits its bioavailability. By using nanotechnological methods , a drugs bioavailability can be enhanced [13].

Vitex negundo Linn., and *Vitex peduncularis* Wall. ex Schauer belong to the family Verbenaceae. These two plants have the potential to treat diabetes[22,25]. *Vitex negundo*, sometimes referred to as the "five-leaved chaste tree," is a small, aromatic tree or shrub that can reach a height of three meters or less. Stems are quadrangular and densely whitish, with tomentose branchlets [15]. The genus vitex, which belongs to the family Verbenaceae, is comprised of about 250 species, which are generally shrubs or small trees , mainly occurring in tropical to temperate regions worldwide[17]. It is commonly found near water bodies and moist and humid places. It can be found growing in wastelands and mixed open forests. The plant is broadly dispersed all through India up to an elevation of 1500 meters conjointly in Mediterranean nations and Central Asia [15,16]. According to text like the Charaka Samhita, it has been used in Ayurveda to treat various ailments such as inflammatory conditions, joint pain, and digestive issues. Similarly, it has been utilized in Unani medicine and Traditional Chinese Medicine (TCM) for its potential benefits, including anti-inflammatory and analgesic effects its historical use across multiple systems underscores its significance in traditional healing practices[18] . The different parts of the plant – leaves , stems , bark ,

flowers, and roots contain a variety of secondary metabolites like negundin , nishindaside, vitexin , vitedoin , and mussaenosidic acids, which contribute to its therapeutic effects[21]. The research into its in vitro alpha-amylase (α-amylase) and alpha-glucosidase (α-glucosidase) inhibitory activities is particularly interesting because these enzymes are essential for the absorption of glucose and the digestion of carbohydrates . Alpha-amylase breaks down starches into sugars, while alpha-glucosidase further breaks down these sugars into glucose. Inhibiting these enzymes can help manage blood sugar levels, which is especially relevant for diabetes management. Studies that explore the inhibitory effects of *Vitex negundo* on these enzymes could provide valuable insights into its potential as a natural remedy for glucose regulation[22,28].

Vitex peduncularis Wall. ex Schauer is a perennial tree growing up to a height of about 5 – 15 meters . Its leaves are generally 3-foliolate . It is widely distributed throughout the Eastern Himalaya and tropical areas of India . It is commonly found in wet areas like moist deciduous forests along water streams and rocky areas at an altitude up to 1000 meter[19]. The plant of *Vitex peduncularis* is a source of various phytochemicals or bioactive agents like iridoid , vitexin , peduncularaside , anguside, flavonoids , and triterpenoids[23]. The Presence of these wide varieties of secondary metabolites in *V. peduncularis* has been proven to be effective against several diseases including diabetes mellitus [19,24,25].

Recent studies have suggested that oxidative stress and inflammation are closely associated with diabetes mellitus[20]. During an oxidative stress condition , there is an increase in the reactive oxygen species (ROS) like superoxide (O_2), hydroxyl (OH), and peroxyl (OOH,ROO). The excess of reactive oxygen species will lead to the pathogenesis of several diseases, including inflammatory disease[26] . Which is caused by the activation of pro-inflammatory enzymes such as lipoxygenase, hyaluronidase, xanthine oxidase, and inducible nitric oxide synthase that are activated by excess of generated ROS[27]. Lipoxygenase enzymes play a vital role in the biosynthesis of leukotrienes (hydroperoxides), which give rise to several inflammatory diseases[29]. The hyaluronidase enzyme was responsible for degranulation of mast cells, which results in the release of inflammatory mediators, leading to the pathogenesis of many diseases, including rheumatoid arthritis, and when inflammatory cells are activated, that causes nitric oxide synthase to produce an excess of nitric oxide, which will lead to inflammation. Xanthine oxidase causes a gout . It is a type of metabolic disease that is linked with inflammation[27].

These all four pro-inflammatory enzymes were responsible for the pathogenesis of inflammation and also some inflammation associated diseases. So we need to inhibit these enzymes, and then we are able to inhibit the prevalence of diabetes and also some other diseases that are associated with oxidative stress and inflammation.

In the present study , due to the rapid growth of metabolic disorders like diabetes, *V.negundo* Linn. and *V.peduncularis* Wall. ex Schauer plant extracts are used for the green synthesis of nanoparticles and the analysis of their antidiabetic activity.

This study will help in the nanoformulation of a drug that is beneficial in the treatment of diabetes.

PROBLEMS TO BE INVESTIGATED

Antidiabetic , antioxidant , and anti-inflammatory activities of synthesized silver nanoparticles, from the leaves of *Vitex negundo* and *Vitex peduncularis.*

OBJECTIVES OF THE INVESTIGATION AND MOTIVATION

Following are the broad objectives of my study :

1. Collection of the plant and its authentication by literature reviews , and from the Department of Botany , Ranchi University, and the plant specimens will be preserved at the herbarium of Botanical Survey of India (BSI) Lucknow.
2. Extract preparation from leaves of *V.negundo* and *V. peduncularis .*
3. Phytochemical analysis of the prepared extract.
4. In-vitro antidiabetic , antioxidant , and anti-inflammatory activities of the crude extract.
5. Green synthesis and characterization of silver nanoparticles synthesized from the leaf extracts of *V.negundo* and *V.peduncularis*.
6. In-vitro antidiabetic , antioxidant, and anti-inflammatory activities of the synthesized silver nanoparticle .
7. A Comparative study of the antidiabetic, antioxidant and anti-inflammatory activities of crude extract and synthesized silver nanoparticles .
8. Biostatistical analysis.

MOTIVATION BEHIND THE WORK

Diabetes is now emerging as a serious threat to population health. Many people are suffering from this chronic illness. So we need to prevent its prevalence and improve the health status of the population. There are many research works were done on the synthesis of nanoparticles and their applications in various scientific domains, but the antidiabetic activity of silver nanoparticles synthesized from *Vitex negundo* and *Vitex peduncularis* plants was not studied . This study will investigate the antidiabetic activity of silver nanoparticles synthesized from the leaves of *V. negundo* and *V. peduncularis* , and the proposed work, seeing the gap in the study, and becoming very helpful in the nanoformulation of novel drugs for treating diabetes.

HYPOTHESIS

The present study supports the antidiabetic properties of silver nanoparticles synthesized from *Vitex negundo* and *Vitex peduncularis* crude extract , which further

helps in making nanomedicine because of their therapeutic potential for the treatment of diabetes mellitus.

RESEARCH DESIGN & METHODOLOGY

Following are the methodologies that are used to complete the objective of my proposed work :

1.Literature survey

2.Collection of material :-

a) Collection of plants from Ranchi district (JHARKHAND).

b) Identification and authentication of collected plants by literature reviews , and from the Department of Botany, Ranchi University, and the plant specimens will be preserved at the Herbarium of Botanical Survey of India (BSI) , Lucknow.

3.Laboratory based work:-

a) ***Extract preparation and preservation***

i. Extract preparation from the leaves of the plant will be done according to the (Rani *et al.,* 2023) method with some modifications.

ii. Prepared extract will be preserved at optimum temperature in the laboratory for further analysis.

b) ***Phytochemical analysis of crude extract***

Phytochemical analysis will be done according to (Nortjie *et al.,* 2022) method with slight modifications.

i. Qualitative screening of the prepared extract, including carbohydrates, alkaloids, phenols, flavonoids , tannins , terpenoids , steroids, and glycosides, shall be carried out in compliance with an established process.

ii. Quantification of total phenol and flavonoid content.

c) ***Antidiabetic-assay of crude extract***

- In- vitro α- amylase and α- glucosidase inhibition assay of crude extract will be Performed according to (Perumalsamy & Krishnadhas ,2022) with slight modifications to determine the antidiabetic activity of the crude extract .

d) ***Antioxidant -assay of crude extract***

- In- vitro antioxidant activity will be performed according to the (Kumari *et al.,* 2023) method with some modifications to determine the free radical Scavenging activity of the crude extract .

e) ***Anti-Inflammatory-assay of crude extract***

- In-vitro pro-inflammatory enzyme inhibition of crude extract will be performed by Arachidonate 5-lipoxygenase (A5-LOX) inhibitory assay, Hyaluronidase inhibitory assay, Xanthine oxidase inhibitory activity and Nitric oxide production inhibitory activity according to (Perera et al., 2018).

f) ***Gas chromatography -mass spectrometry (GC-MS) analysis.***

The bio-active compounds of the extract will be determined by the GC-MS analysis (Gomathi *et al.,* 2013).

g) ***Green synthesis of nanoparticles using leaf extracts of plants .***

Silver nanoparticles will be synthesized using the methods of (Rani *et al.,*2023) and (Zargar *et al.,*2011), with some modifications.

h) ***Chracterization of nanoparticles synthesized from extract:-***

The following are some techniques that were used to characterize the synthesized nanoparticles –

i. **UV-VIS Spectroscopy-** This method will be used for the initial characterization of synthesized nanoprticles . In order to verify the creation and stability of nanoparticles, it entails monitoring the absorption of light at various wavelengths (Nagaraja *et al.,*2022).

ii. **FT-IR analysis-** It will be used to find the functional groups in plant extracts that were responsible for the reduction of metal ions during the synthesis of metal nanoparticles (Nagaraja *et al.,*2022).

iii. **XRD analysis**- The XRD technique will be used to validate the crystalline structure of the synthesized nanoparticles (Kumari *et al.,* 2023).

iv. **SEM-EDX analysis**- SEM and EDX studies will be used to examine the surface morphology and basic compositions of the synthesized nanoparticles (Patra *et al.,* 2019).

i) ***Antidiabetic- assay of the synthesized nanoparticle.***

- In – vitro α- Amylase and α-Glucosidase inhibition assays will be carried out in accordance with (Perumalsamy & Krishnadhas,2022) , with some modifications to determine the antidiabetic activity of synthesized nanoparticles.

j) ***Antioxidant-assay of the synthesized nanoparticle.***

- In-vitro antioxidant activity will be performed according to the (Kumari *et al.,*2023) method with some modifications to determine the free radical scavenging activity of the synthesized nanoparticle.

k) ***Anti-Inflammatory- assay of the synthesized nanoparticle.***

- In-vitro pro-inflammatory enzyme inhibition of synthesized nanoparticle will be performed by Arachidonate 5-lipoxygenase (A5-LOX) inhibitory assay, Hyaluronidase inhibitory assay, Xanthine oxidase inhibitory activity and Nitric oxide production inhibitory activity according to (Perera *et al.,* 2018).

4. A Comparative study of the in-vitro antidiabetic , in-vitro antioxidant, and in-vitro anti- inflammatory activities of crude extract and synthesized nanoparticles.

5. Biostatistical analysis

MATERIALS AND METHODS

Plant required –

Vitex negundo, *Vitex peduncularis*.

Apparatus required –

Test tubes, test tube holder, test tube stand, beaker, conical flask, sample container, measuring cylinder, whatman filter paper, funnel, falcon tubes, cotton plug, spatula etc.

Various chemicals required –

Methanol, Ethanol, Ferric chloride, Chloroform, conc. Sulphuric acid, Hydrochloric acid, Sodium hydroxide, Fehlings reagent, Mayer's reagent, Silver nitrate, distilled water, arachidonate 5-lipoxygenase (A5-LOX), linoleic acid, baicalein, hyaluronic acid potassium salt, hyaluronidase, calcium chloride, p-Dimethylaminobenzaldehyde (PDMAB), sodium borate, tannic acid, xanthine oxidase, xanthine, allopurinol, etc.

Machine required –

Refrigerator , weighing machine , hot air oven , Water bath , Electric grinder , Soxhlet extractor , Rotary evaporator , Magnetic stirrer , Incubator , Centrifuge etc.

Place of work

Laboratory - based work will be done in the University Department of Botany , Ranchi University , Morabadi.

SIGNIFICANCE 0F THE STUDY

The following are some significances of this study :

1. This study will help to reveal the antidiabetic properties of *V.negundo* and *V.peduncularis,* and will give a comparative evaluation of the in-vitro

antidiabetic, antioxidant , and anti-inflammatory activities of the crude extract and synthesized silver nanoparticles.

2. The study of the antidiabetic activity of nanoparticles will be beneficial for treating diabetes mellitus and its associated complications.
3. This study will affirm the superior biological capability of nanoparticles for target drug delivery and targeted drug therapy.

IT'S RELEVANCE TO PRESENT DAY PROBLEMS AND NEED OF THE SOCIETY

1. Diabetes is a persistent disease; therefore, the patients who suffer from this disease need ongoing medication and self-management education to treat its associated acute complications and minimize the risk of its long-term complications .
2. According to the International Diabetes Federation (IDF) Diabetes Atlas, 10th edition, about 537 million adults worldwide are living with diabetes . In the year 2021, it is reported that about 8.4 million people have Type 1 diabetes , 460 million suffer from Type 2 diabetes, and 21.1 million get diagnosed with gestational diabetes worldwide . Whereas in India , 8.6 lakh people had Type 1 diabetes , 77 million had Type 2 diabetes and 5 million had gestational diabetes .India has the second-largest number of diabetics worldwide . A survey by the National Family Health (2021) showed that the prevalence of diabetes in Jharkhand was 5.8% in urban and 5.2% in rural areas.
3. The global prevalence of diabetes has been rising steadily. Growing populations, ageing populations, urbanisation, physical inactivity, and obesity are the main risk factors for diabetes mellitus prevalence.
4. Currently , there are many therapeutic options available for the treatment of diabetes , but herbal medicines are recommended mostly due to their efficiency, affordability, and lack of negative side effects. The information and parameters that are provided by many pharmacopoeias can be used to maintain the quality and standardise the processes used in the identification / authentication of herbal products and their derivatives . The proposed work will help in the treatment of diabetes through nanoformulation of herbal drugs .
5. Nanoparticles are synthesized using traditional synthetic methods of physico- chemical method, which are developed by many researchers . This physicochemical method which is use to synthesize nanoparticles is potentially hazardous to the environment . In this conventional method there is usage of toxic and expensive chemicals with hazardous organic solvents . These toxic materials are present on the surface of nanoparticles, which are responsible for the toxic nature of the nanoparticles . The disposal of harmful organic solvents also creates environmental problems .
6. Currently, many research groups are focusing on the biosynthesis of metal nanoparticles because of their eco-friendly nature and because it is an economical, green, and easy method for the synthesis of nanoparticles.

7. Biosynthesized nanoparticles are biocompatible ; this property makes nanoparticles a better alternative, involving biomolecules as reducing and capping agents compared with nanoparticles , which are synthesized by traditional synthetic methods .
8. The other advantage of using this green , environmentally friendly method is that it has a safe protocol that includes a mild reaction conditions , a simple reaction setup , and the use of non – toxic green solvents like glycerine and water. This method shows compatibility for further biomedical research and pharmaceutical applications.

FUTURE WORK

- This study will help to flourish the use of cheap and biologically eco-friendly method for the synthesis of nanoparticles which is efficient for future work.
- This study intends to reveal the antidiabetic activity of nanoparticles in a simple and fast way .
- By using nanoparticles to treat diabetes, there is fresh promise for improving population health and life expectancy, which will help with the development of innovative medication formulations.
- For future work , the green synthesized nanoparticles can be subjected to develop into an efficient drug delivery system as they are smaller in size.
- This study will help in further work , which is necessary to elucidate in detail the mechanism of action of nanoparticles at the cellular and molecular level .

REFERENCE

1. Bayda S,Adeel M, Tuccinardi T, Cordani M , Rizzolio F , [2020]. The history of nanoscience and nanotechnology: From chemical- physical applications to nanomedicine.Molecules 25[1]:1-15.
2. Murthy S.K,[2007].Nanoparticles in modern medicine state of the art and future challenges.International journal of nanomedicine 2[2]:129-141.
3. Thakkar K.N, Mhatre S.S, Parikh R.Y,[2010]. Biological synthesis of metallic nanoparticles. Nanomedicine 6[2]:257-262.
4. Altammar K.A,[2023]. A review on nanoparticles: characteristics , synthesis, applicatons , and challenges. Front Microbiol 14:1155622.
5. Patra J.K, Das G, Shin H.S, [2019]. Facile green biosynthesis of silver nanoparticles using *Pisum sativum* L. outer peel aqueous extract and its antidiabetic, cytotoxicity,antioxidant , and antibacterial activity . International journal of nanomedicine 14:6679-6690.
6. Patil S.P , Kumbhar S.T, [2020]. *Vitex negundo* assisted green synthesis of metallic nanoparticles with different applications : a mini review. Future journal of pharmaceutical sciences 6:90.
7. Reddeppa M, Reddy R. C.K, RaJ V.P, Rani T.S, [2019]. Green synthesis of copper nanoparticles : Evaluation of catalytic and antibacterial activity. Asian journal of chemistry31[3]:622-626.
8. Kumari B.V,Mani R, Asokan B.R, Balakrishnan K, Ramasamy A, Parthasarathi R, Kandasamy C, Govindaraj R, Vijayakumar N, Vijayakumar S,[2023]. Green synthesised silver nanoparticles using *Anoectochilus elatus* leaf extract : characterization and Evaluation of antioxidant, anti-inflammatory, antidiabetic , and antimicrobial activities .Journal of composites science 7,453.
9. Mallmann E.J,Cunha F.A, Castro B.N.M.F, Maciel A.M, Menezes E.A, Fechine P.B.A,[2015]. Antifungal activity of silver nanoparticles obtained by green synthesis. Rev Inst Med Trop Sao Paulo 57[2]: 165-167.
10. Gopalakrishnan R,Loganathan B,Dinesh S, Raghu K,[2017].Strategic green synthesis, characterization and catalytic application to 4-nitrophenol reduction of palladium nanoparticles. J Clust Sci 28:2123-2131.
11. Sharma J.K, Akhtar M.S, Ameen S, Srivastva P, Singh G,[2015]. Green synthesis of CuO nanoparticles with leaf extract of *Calotropis gigantea* and its dye- sensitized solar cells applications.J Alloys Compd 632:321-325.
12. Raza M.A,Farwa U,Mumtaz M.W,Kainat J, Sabir A, AL-Sehemi A.G,[2023]. Green synthesis of gold and silver nanoparticles as antidiabetic and anticancerous agents . Green chemistry letters and reviews 16,no.1, 2275666.
13. Nagaraja S, Ahmed S.S, B.R.B, Gaudanavar P, M.R.K, Fattepur S, Meravanige G, Shariff A, Shiroorkar P.N, Habeebuddin, Telsang M, [2022]. Green synthesis and characterization of silver nanoparticles of *Psidium guajava* leaf extract and evaluation for its antidiabetic activity . Molecules 27,4336.

14. Marcella S, Tollamadugu N.V.K.V.P,[2018].Nanotechnological approaches for the development of herbal drugs in treatment of diabetes mellitus – a critical review. IET Nanobiotechnology 12[5]:549-556.
15. Suva M.A , Sharma N,[2014]. A brief review on *Vitex negundo* Linn:Ethnobotany, phytochemistry and pharmacology . Inventi journals (P) Ltd [1]:1-4.
16. Ullah Z, Ullah R, Haq Ali Shah A, Ahmad I, Haider S , [2012]. Phytochemical and biological evaluation of *Vitex negundo* Linn: A review .International journal of pharmaceutical sciences and research 3[8]:2421-2431.
17. Diaz F,Lee D, Tan G.T, Kardono L,[2003]. Cytotoxic flavone analogues of vitexicarpin, a constituent of the leaves of *Vitex negundo*. Journal of natural products 66:865-867.
18. Sagitha T , Romauld S.I, Devi P.B, Vivek P, Kumari N.K, Sudharsan M.S, Anbarasu M,[2023]. Antibacterial, antidiabetics and larvicidal activity of *Vitex negundo* and *Ocimum basilicum* L. extracts. Oxidation communications 46[1]:46-55.
19. Sundar S.D, Sreya D, Manosi D, Kumar R.K, Das D, Jayram H, [2016]. Pharmacognostical profiling on the leaf of *Vitex peduncularis* wall.ex Schauer (charaigorh).International journal of research in ayurveda and pharmacy 7[6]:25-32.
20. Oguntibeju O.O, [2019].Type 2 diabetes mellitus, oxidative stress and inflammation:examining the links. International journal of physiology, pathophysiology and pharmacology 11(3):45-63.
21. Basri F, Sharma H.P, Firdaus S, Jain P, Ranjan A, [2014]. A review of ethnomedicinal plant – *Vitex negundo* Linn. International journal of advanced research 2[3]:882-894.
22. Mathavi P, Nethaji S, [2019]. Screening of phytochemicals and antidiabetic activity of *Vitex negundo* leaves . Pramana research journal 9[2]:78-84.
23. Rawat S, Baniwal S,[2023].A review on world's potential anti-malarial plants and their phytochemicals. Annals of science and allied research 1[1]:116-123.
24. Haque Md.E, Rani M, Jahan A, Islam Md.A, Alam Md. A ,[2018]. An overview study on chemical constituents and biological activities of *Vitex peduncularis* wall . MOJ bioorganic & organic chemistry 2[1]:6-9
25. Deb C.R, Sharma T.I , Jamir N,[2023]. Ethno-medicinal anti-diabetics plants of Northeast India: A review. Journal of pharmacognosy and phytochemistry 12[3]:86-110.
26. Sakat S.S, Juvekar A.R, Gambhire M.N, [2009].In-vitro antioxidant and anti-inflammatory activity of methanol extract of *Oxalis corniculata* Linn. . International journal of pharmacy and pharmaceutical sciences 2(1):146-155.
27. Perera H.D.S.M, Samarasekera J.K.R.R, Handunnetti S.M, Weerasena O.V.D.S.J, Weeratunga H.D, Jabeen A, Choudhary M.I, [2018]. In vitro pro-inflammatory enzyme inhibition and anti-oxidant potential of selected sri lankan medicinal plants. BMC complementary and alternative medicine 18:271.
28. Perumalsamy R , Krishnadhas L, [2022]. Anti-diabetic activity of silver nanoparticles synthesized from the hydroethanolic extract of *Myristca fragrans* seeds. Applied Biochemistry and Biotechnology. https://doi.org/10.1007/s12010-022-03825-8.

29. Paun G, Neagu E, Albu C, Savin S, Radu G.L,[2020]. In vitro evaluation of antidiabetic and anti-inflammatory activities of polyphenolic-rich extracts from *Anchusa officinalis* and *Melilotus officinalis*. ACS omega 5:13014-13022.